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NOTES ON THREE HEMLOCK FUNGI

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(WITH PLATES 10 AND 11 AND 1 TEXT FIGURE)

Among the large number of fungi described by Peck as new species was one found by him on *Tsuga canadensis*. It was the first of two species which he described under his new genus *Gelatinosporium*.[1] Several years later he found an ascocarpic fungus on *Abies balsamea*. He described this one as a new species of *Cenangium*, *C. balsameum*.[2]

Early in November 1931, our attention was called to a few small hemlock trees at Scarsdale, N. Y., which were in bad condition. They had been planted in this place a few years previously. Some of the trees had recently died and branches on others were losing their leaves as though infected with some fungus blight. As a rule no fungus fruiting bodies were present, but certain dead branches that had been pruned off were found thickly dotted with conidial fructifications (PLATE 10, *b*) corresponding very well with Peck's *Gelatinosporium abietinum*, as typified by a specimen collected on hemlock by Peck at Greenbush, N. Y., and now at The New York Botanical Garden as no. *2488* of the Ellis collection. It is probably a part of the type.

On the lower ends of some of the same branches of the Scarsdale hemlocks bearing the *Gelatinosporium* were many mature ascocarps of Peck's *Cenangium balsameum* (PLATE 10, *a*). Such an association was noted by Peck[3] for *C. balsameum* var.

[1] Ann. Rep. N. Y. State Mus. **25**: 84. 1873.
[2] Ann. Rep. N. Y. State Mus. **38**: 101. 1885.
[3] Ann. Rep. N. Y. State Mus. **43**: 40. 1890.

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abietinum. Thaxter [4] refers to Peck's notice of this association in commenting on specimens of this species in no. *102a*, Reliquiae Farlowianae, collected on *Tsuga canadensis*. Nos. *102b* and *102c* are labeled to indicate that *Gelatinosporium abietinum* is the pycnidial stage of *Cenangium balsameum*. There are several morphological features that would have suggested such a connection. The pycnidia and ascocarps are both blackish-olive colored on the exterior; both kinds of fruit bodies are rather tough gelatinous when wet; the tissues within both tend to be light olivaceous or greenish yellow; the conidia usually show about three septa, as do the ascospores at maturity; both kinds of spores are somewhat curved (PLATE 11, *c*, *e*). These similarities afford another example of the sort of parallelisms which Orton [5] pointed out as frequently indicating genetic relationships between conidial and ascocarpic fructifications. Such parallelisms have also been used by Arthur and his colleagues in predicting connections between rust spore-forms.

The conidia from a specimen of the form genus *Myxosporium*, to be noted later, are much broader than are those of *Gelatinosporium* but they also show three or four septa at maturity. *Myxosporium* has been placed among the Melanconiales with 1-celled hyaline spores. The connection between species of *Myxosporium* and *Dermatea* and the advisability of referring *Cenangium balsameum* to *Dermatea* will be noted later.

SINGLE SPORE CULTURES

Ten cultures obtained from single ascospores of this "*Cenangium*" on hemlock, and fifteen cultures from single conidia of the *Gelatinosporium* on the same branches, all showed similar characters and the same developmental stages, thus positively confirming the connection suspected by Peck [3] from the proximity of the two kinds of fruit bodies on the same branches.

Growth is slow, especially on Czapek's medium, which it turns brown. Few fruit bodies develop on this medium. A fine whitish mycelium with little aërial growth develops on corn meal agar. A few spore-bearing structures are always formed. On

[4] Mycologia **14**: 99–103. 1922.

[5] Orton, C. R. Structural parallelism between spore-forms in the Ascomycetes. Mycologia **7**: 21–27. 1915.

dextrose agar the growth at the surface is compact, powdery, and at first cream colored, then isabelline. Fruit bodies are rarely formed on this medium. Potato-dextrose agar seems to be more favorable for normal growth of the fungus. Numerous fruit bodies which show some of the grayish olivaceous color so characteristic of this species are always matured.

Microconidia. At the end of about one month in culture on corn meal agar, small whitish to yellowish-tan tubercle-like bodies are formed, either at the surface or beneath the agar. These bodies are more in the nature of small fleshy stromata. A cavity (PLATE 11, *b*) in which quantities of allantoid to hamate microspores (PLATE 10, *e*) come to lie is formed through excess of peripheral growth of wall elements. Mixed in with the microconidia one usually finds a few septate macroconidia. The fruiting structure turns brown as it ages.

Macroconidia. In some cases only an incipient micropycnidial primordium with a few microspores is formed in agar cultures. Extending from this point well below the surface, a canal, lined with macroconidia, is formed through the agar (PLATE 10, *c*). This simulates an ostiolar structure, but these conidia are cut off directly from hyphal branches before any definite fruit body is apparent (TEXT FIGURE 1, *b*). A little later the sporogenous elements do become organized into a wall structure which increases in size peripherally and then the conidia are cut off from definite sporophores which now line the irregular cavity (TEXT FIGURE 1, *d*). Fertile knobs of sporogenous tissue protrude inwardly so that a section may suggest a multilocular structure (PLATE 10, *c*), like that of a *Fusicoccum*. The cavity rounds out, however, to become a well organized pycnidium with an opening above, through which the spores are discharged. The ostiole is not definitely limited by papillary growth in the way one finds it in a *Phoma*, for example.

The fruit bodies which are formed so abundantly on potato-dextrose agar in cultures two or three months old, are covered with a fine soft puberulent growth such as one sees on young ascocarps of a *Cenangium*. They have the same form taken by the young microconidial structure shown in plate 11, *b*. Very likely the micropycnidium becomes a macropycnidium directly.

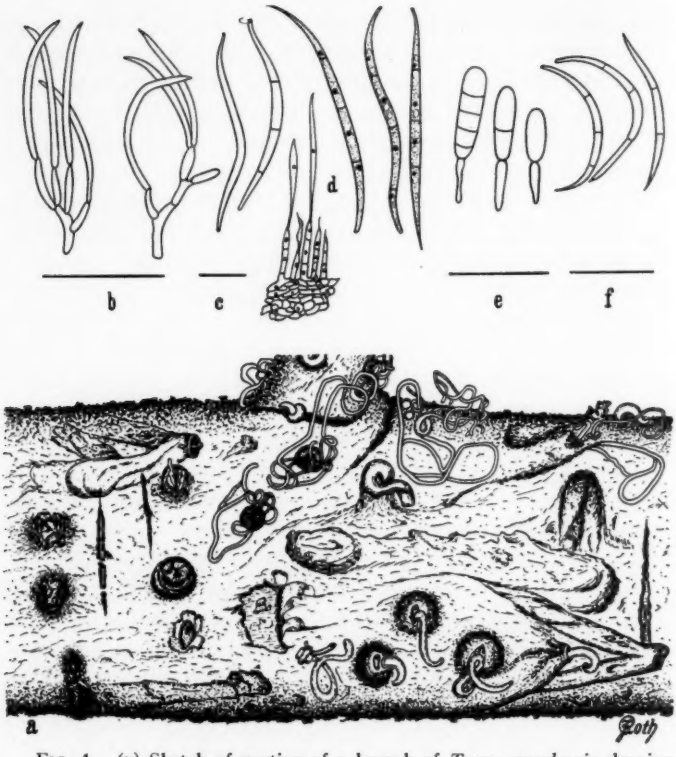


FIG. 1. (*a*) Sketch of portion of a branch of *Tsuga canadensis* showing discharge of spores of *Gelatinosporium abietinum* in the form of cirrhi; one ascocarp of *Dermatea balsamea* (Peck) Seaver at the left. (*b*) Conidia arising on sporophores (taken from culture.) (*c*) Conidium from nature, septa not always readily seen. (*d*) Conidia and their sporophores from section of pycnidium on a branch. (*e*) Conidia of *Myxosporium abietinum* Rost. (?) from the same hemlocks. (*f*) Conidia of *Micropera Abietis* Rost. from Orton's specimen.

Later on some of these structures develop a small hole at the center of the dome through which typical macroconidia exude in long cirrhi. Not all of these bodies, however, produce masses of conidia in this way. In fact the central cavity seems to close up in some of them without producing many conidia and then the structures have a strong resemblance to young ascocarps.

PYCNIDIA ON BRANCHES

The name *Gelatinosporium* was suggested by the gelatinous spore masses one sees within and about the pycnidia in moist weather (PLATE 10, *b*). Ordinarily the conidia ooze out in long whitish to light greenish cirrhi. Not infrequently several spore horns come out close together (TEXT FIGURE 1, *a*). Sections show that several pycnidia may be formed together, possibly from the same stromatic center. A section of one-half of a pycnidium on a hemlock branch is shown in plate 10, *d*, and a portion of the same structure more highly magnified in plate 11, *a*. These figures prove that there is a very definitely organized wall structure with an opening at the apex. The opening is merely a break in the overlying wall, however. The conidiophores are at first rather long and slender, varying from one to four cells in length (TEXT FIGURE 1, *d*). Each cell has a single nucleus. The tip spins out to a fine point from which the conidia grow out, a new one forming after the old one has broken away. New sporogenous elements arise as the cavity increases in diameter.

The conidia are at first merely allantoid, "more or less curved," Peck says, but later there occurs a peculiar change which is apt to give them a sort of twisted sigmoid appearance. This is brought out in the photograph shown in plate 11, *c*. Under high power one end of the conidium is usually out of focus. The conidia are rather brittle and tend to break off at a septum. The septa which are formed before the spores are mature do not show well in the photographs. Each cell contains a single nucleus (TEXT FIGURE 1, *d*). The "rows of nuclei" mentioned by Peck are merely little portions of cell contents that round up, especially if one puts the twigs in a damp chamber for a few days.

PATHOGENICITY

Experiments to determine to what extent this fungus is responsible for the dying out of the hemlocks referred to are being carried on. Young hemlocks that had become dormant were brought into the greenhouse and sprayed with spore suspensions. No blighting effects have become manifest as yet. Dr. Glenn Hahn of the U. S. Department of Agriculture is engaged in a study of a number of fungus parasites of conifers.

He writes in part as follows: "We are greatly interested in what you have to say concerning *Cenangium balsameum*. During the past summer I collected a *Cenangium*-like fungus on balsam fir in Maine, which was apparently associated with a die back of the terminal shoots. We found *Micropera Abietis* Rost. associated with this fungus. Unfortunately we were unable to secure cultures of either." This raises the question as to whether *M. Abietis* may not be identical with *Gelatinosporium abietinum*.

There is in the herbarium of The New York Botanical Garden a specimen collected on *Abies balsamea* by C. R. Orton and identified as *Micropera Abietis* Rost. by L. O. Overholts. To judge from its conidia (PLATE 11, *d*) it is not Peck's *Gelatinosporium abietinum*. The conidia are semicircular-arcuate as stated by Rostrup, but they are somewhat longer, 50–60 μ. They are usually about 3-septate. The conidia of our *Gelatinosporium*, which are shaped like those from the Peck specimen mentioned previously and also like the Farlow specimen (no. *102b*), are usually about 3-septate and 70–80 μ long when mature. The septa are not readily seen and might have been easily overlooked by the original authors of both species. Peck's sketch on the Greenbush specimen referred to above actually shows spores with two septa. One finds a few microspores in mounts from Orton's specimen (PLATE 11, *d*, left). Dr. Orton says in a letter: "I collected the specimen in question at Hollywood, N. Y. The disease was in evidence over quite an area in that region, and appeared to be very destructive on young balsam firs at that time." He further referred to an article by Fron,[6] who had found this fungus in fir plantations of the Jura. "He (Fron) was of the opinion that it was the same as Rostrup's fungus which was first described on *Abies pectinata* in Denmark." Fron had compared his fungus with the type of Rostrup's *Micropera Abietis*, and accounted for the smaller size of the conidia in the type specimen by the immaturity of that material. Fron's figure of a spore is very similar to the photograph of spores from Orton's specimen shown in our plate 11, *d*. These conidia, 50–60 μ long, were certainly mature. Fron's conidia were even

[6] Fron, M. G. Note sur le *Micropera Abietis* Rostrup. Bull. Soc. Myc. Fr. **24**: 169–171. 1908.

longer, 60–70 μ. He also shows that one conidium is formed after another from the tip of the same sporophore. They are cut off in the same way in our *Gelatinosporium*. Very likely there is another species of *Dermatea* ("*Cenangium*") with *Micropera Abietis* Rost. as its asexual stage just as believed by Rostrup. It is not probable that cultural studies would prove this to be the same as Peck's species. In any event Peck's specific name, *abietinum*, would still hold. Whether one calls it a *Micropera* or a *Gelatinosporium* is of little matter when the ascocarp stage is known. *Micropera* is an older genus name, so that *Gelatinosporium* would become a synonym if *M. Abietis* is a *Micropera* as typified by *M. drupacearum*, which is said to be the asexual stage of *Dermatea Cerasi*. Gregor [7] remarks relative to Rostrup's belief that *Micropera Abietis* is the pycnidial stage of *Dermatea eucrita* (*D. livida*): "Neither *Stilbella* nor *Micropera*, however, shows any resemblance to the true imperfect stage of *D. livida*." She had proved this to be *Myxosporium abietinum* Rost., which has an acervulus fruit body, while it is said *Micropera* has a true pycnidium. There may be still another *Dermatea* on *Abies*.

Dr. F. J. Seaver is now monographing the genus *Dermatea*. The writer is not particularly concerned with the taxonomic aspects of the question, but on consulting with Dr. Seaver as to the identity of the *Gelatinosporium* referred to previously, it was found that he had already placed *Cenangium balsameum* of Peck in the genus *Dermatea* under **Dermatea balsamea** (Peck) Seaver, comb. nov., and had considered *Gelatinosporium Abietis* its asexual stage, following the statement on the Farlow specimens (Reliq. Farlow. *102b*). This would answer the objection raised by Gregor; and the statement by Dr. Hahn in the quotation from his letter relative to his finding *Micropera Abietis* associated with a *Cenangium*-like fungus is thus understandable.

MYXOSPORIUM ABIETINUM ROST.?

On the same branches of hemlock which had been showing so many pycnidia of *Phomopsis occulta*, to be noted below, were

[7] Gregor, Mary J. F. A comparative study of growth forms within the species *Dermatea livida* (B. & Br.) Phill. Ann. Bot. **45**: 73–90. 1931.

found some fruiting bodies which were originally taken to be a *Hendersonia* or a *Coryneum*. The spores which are borne on long thick stalks (PLATE 11, *f*, and TEXT FIGURE 1, *e*) are at first 1-celled and hyaline. Later they become somewhat colored, light olivaceous or slightly brownish and 3- to 4-septate. The number of septa is variable, however. Gregor [5] has given us a detailed account in which the relationship between *Dermatea livida* (Berk. & Br.) Phillips and *Myxosporium abietinum* Rost. is discussed. Cultures from single conidia of our fungus suggest that it is a good *Myxosporium* and probably *M. abietinum*. The conidia are about 25–37 μ long and 12–13 μ wide. Practically all of the mature spores show three or four septa and then tend to germinate in the old cultures. Microspores of the form and size (5–6 $\times$ 1.5–2 μ) of those figured by Gregor are also produced in our cultures. Dark colored tubercle-like to flatly conical fruit bodies are formed either on or beneath the surface of the agar medium. In some cases at least, the conidia are formed in a cavity in a structure which breaks open so that the conidia spread out in masses. Some might prefer to call these structures pycnidia. In any event in its morphology and cultural characters this species is quite distinct from *Gelatinosporium* and it can not possibly be connected with *Dermatea balsamea* (Peck) Seaver, although the *Myxosporium* conidia do more nearly resemble ascospores of *Dermatea*.

DIAPORTHE CONORUM

Certain small branches of the hemlocks which showed some browning of leaves were put in damp chambers. Some time later many more leaves began to turn brown near the point of attachment and, shortly after, they would fall off showing a *Phomopsis* pycnidium at this point. Similar pycnidia soon developed not only on the branches but, finally, directly on the leaf blades. This fungus was certainly growing vigorously on leaves and twigs that were still green. Dr. Hahn, who identified this fungus as *Diaporthe conorum*, of which *Phomopsis occulta* is the pycnidial stage, says that it may occur as a secondary parasite on conifers weakened by unfavorable growth conditions. Many single spore cultures were obtained by germinating the

Phoma type of spores, but the stylospores did not germinate. No blighting effects followed directly the spraying of spore suspensions on dormant hemlocks brought into the greenhouse.

The writer is indebted to Miss Marjorie Swift, who made the single spore cultures and slide preparations for this study, and to Dr. Glenn Gardner Hahn, who identified the *Phomopsis*, and is engaged in making a further study of the pathogenicity and taxonomic aspects of these fungi.

SUMMARY

Three different species of fungi were found on branches of certain small hemlock trees that were showing signs of some fungus blight. The most conspicuous species was identified as *Dermatea balsamea* (Peck) Seaver (*Cenangium balsameum* Peck). Single ascospore cultures produce micropycnidia. The microspores are of a type somewhat like the stylospores of a *Phomopsis*. Well organized pycnidia with slender flexuose slightly twisted sigmoid macroconidia develop later. This asexual stage is *Gelatinosporium abietinum* Peck and it is essentially a *Micropera*, as indicated by von Höhnel.

The identity of species of the *Myxosporium* found on the same dying branches of the hemlocks is uncertain because the ascocarpic stage was not found. It may be *Myxosporium abietinum* Rost., which has been connected with *Dermatea livida* by Gregor. *Micropera Abietis* Rost. has been reported by several persons as occurring on blighted branches of balsam fir. It appears not to be identical with *Gelatinosporium abietinum*. It probably is connected with some other species of *Dermatea*.

Phomopsis occulta, which is the asexual stage of *Diaporthe conorum*, was found as a secondary parasite on the same hemlocks.

The pathogenicity of the three species of fungi studied is being investigated further.

THE NEW YORK BOTANICAL GARDEN,
BRONX, NEW YORK CITY

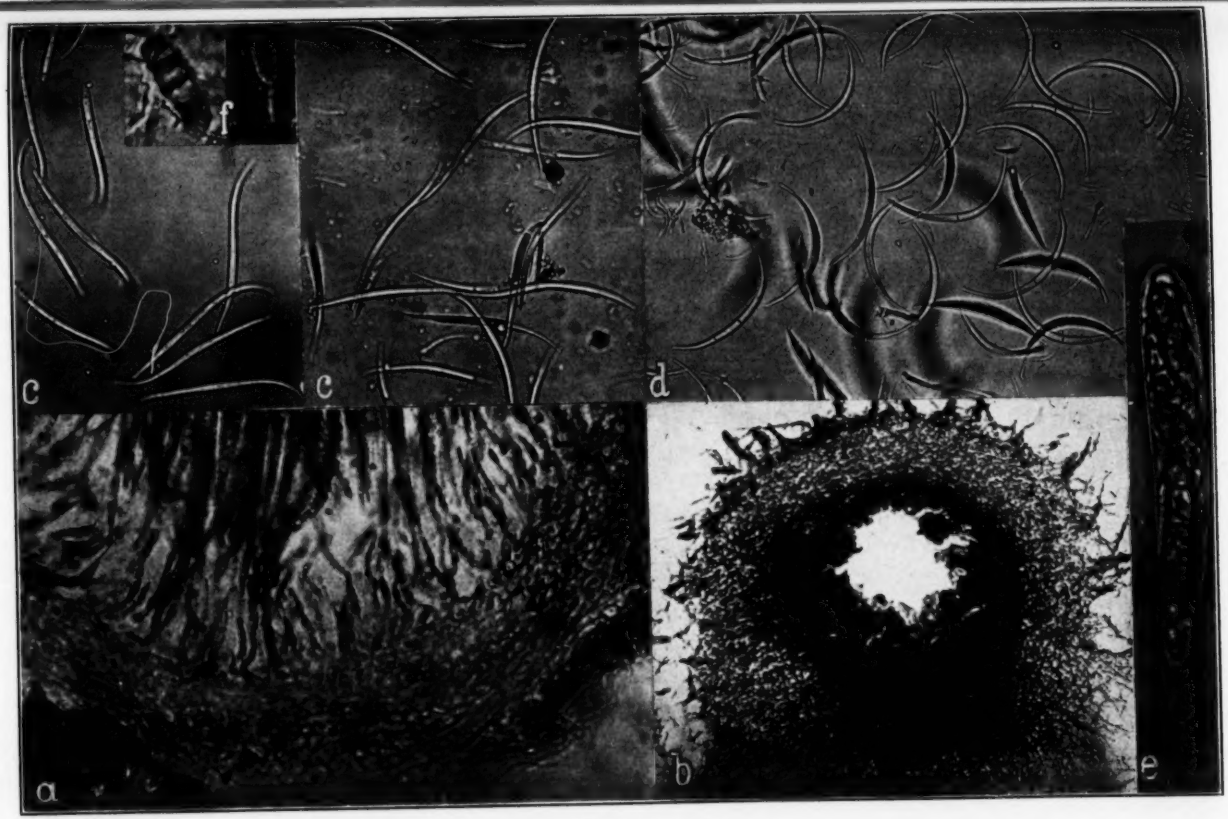
EXPLANATION OF PLATES

PLATE 10

Dermatea balsamea (Peck) Seaver. *a*, Dead branch of *Tsuga canadensis* showing mature ascocarps of Peck's *Cenangium balsameum*. *b*, Dead branch showing pycnidia of the *Gelatinosporium abietinum* stage. Whitish rings are masses of conidia spread out because of moist conditions. *c*, Section of a young pycnidium in agar culture. The mass of macroconidia above seems to be dissolving away the agar to provide an opening. No ostiole is organized. *d*, Section of a mature pycnidium from a hemlock branch. The wall and sporophores are shown better in plate 11, *a*. *e*, Microconidia from cultures. A few macroconidia also present. *f*, Asci. *g*, *h*, Ascospores.

PLATE 11

a, Section of a pycnidium of *Gelatinosporium abietinum* showing wall tissues, sporophores and origin of conidia. Each cell has a single nucleus. *b*, Section of a micropycnidium from cultures; cavity contains masses of microspores. *c*, Twisted, slightly sigmoid macroconidia. *d*, Macroconidia of *Micropera Abietis* Rost. from Orton's specimens. A few microconidia are visible. *e*, Ascus of *Dermatea balsamea* showing 3-septate spores. *f*, Conidia of the *Myxosporium abietinum?* found on the same hemlocks. *c* and *d* are equally magnified for comparison.



SPECIES OF DERMATEA

A PHYSIOLOGICAL AND MORPHOLOGICAL STUDY OF SAPROLEGNIA PARASITICA [1]

BESSIE B. KANOUSE

(WITH PLATES 12 AND 13)

The species of water mold that is found commonly on fish and fish eggs in fish hatcheries and in the fresh water lakes and streams belongs to the genus *Saprolegnia*. This fungus has been the source of a great deal of concern to ichthyologists for many years since it has caused and is continually causing severe losses in the propagation of fish. The fungus is widespread not only in America but also in Europe and much attention has been given to control methods.

Mycologists likewise have been concerned with this fungus and references to it are numerous in the literature. A very interesting early ecological and physiological reference is quoted from Ramsbottom (**10**), who in turn quotes from Spallanzani.[2] An historical review of more recent references has been published by Coker (**2**).

Coker proposed the name *Saprolegnia parasitica* Coker for the fungus associated with fish and fish eggs. The names *S. ferax* (Guith) Thuret. and *S. monoica* Pringsh. have been applied to the fish parasite, but both of these species form oögonia readily

[1] Papers from the Botany Department of the University of Michigan, No. 372, and from the University Herbarium.

[2] "'When a minnow or leech dies under water, even several inches from the surface, and has seldom or never come there but for the purpose of respiration, it will soon be covered with a peculiar kind of long slender mould, while lying at the bottom, as I have repeatedly seen. Various species, some slender, some thicker, others more bushy and slender, grow on different animal or vegetable substances also under water; it then seems more luxuriant, which is a general remark to be made of mould where it imbibes most nutriment. Whence does this mould originate?' He queries whether the mould attacks the fish in the short space of time it spends at the surface of the water. He sees no reason to doubt that mould should not thrive in water just as well as many plants, 'because there are facts which seem to prove the existence of certain species of aquatic mould that are not to be found on terrestrial substances.'"

in culture and also in nature, while *S. parasitica* both in nature and in most conditions of culture remains sterile. It was partly upon this difference that Coker based his specific distinctions.

In this paper the author gives an account of the complete life history of *Saprolegnia parasitica* in which the sexual organs are described for the first time. It is shown that *S. parasitica* differs in important respects from *S. ferax*, *S. monoica*, *S. monoica* var. *glomerata* Tiesenhausen and *S. monoica* var. *vexans* Pieters not only morphologically but also physiologically so that the conception of *S. parasitica* as a distinct species remains sound.

Huxley (**3**) in 1882 described at length a fungus causing an epidemic in the salmon in the streams in England. He was not working with pure cultures of the fungus and he states that he had no proof of the fact that the sexual organs that he observed really belonged to the fungus in question. Coker (**2**) in his description of *S. parasitica* guardedly says, "sexual reproduction not observed so far in our form," and further, "That the species is almost or quite without sexual reproduction is evidenced by the fact that only very rarely have oögonia been reported (and then without proof that they belonged to the parasite) notwithstanding the fact that this species has been more studied perhaps than any other in the family." Apinis (**1**) says of *S. parasitica* in Lettland, "Oögonia nicht entwickelt."

The occurrence of this troublesome fungus in the fish hatcheries in Michigan was brought to the attention of the writer by Dr. Carl L. Hubbs, Director of the Division of Fisheries Research, Museum of Zoölogy, University of Michigan. To him and to members of his staff the writer is indebted for the collections of *S. parasitica* used in this investigation.

The question of sterility in a fungus of this group is always a matter of more than ordinary import. After the well-known investigations of Klebs (**7**), of Kauffman (**6**), and of Pieters (**8**) on species of *Saprolegnia*, it seemed reasonable to infer that, barring the possibility of its being a heterothallic species, oögonia and antheridia could be produced in this species also if the proper environmental conditions could be obtained. The basic hypothesis was that the ability to form reproductive organs was not a lost character, but that it was merely latent and

could be brought into expression by suitable physiological environment. The Klebsian principle of the gradual reduction of food supply as a means of control of vegetative and reproductive manifestations in fungi need only be mentioned as the natural approach to the problem. The results obtained reaffirm once again the correctness of the Klebsian philosophy concerning growth and reproduction in fungi. The results also demonstrate a second principle stressed by Klebs, namely that of specificity. It is shown by the following experiments that, in comparison with other species of the genus, *S. parasitica* has an extraordinarily wide range of adaptability with respect to vegetative and asexual development while its sexual reproduction is more narrowly restricted.

Collections of *S. parasitica* were secured from the following sources: From a sunfish taken from a small lake, Ann Arbor, November 1, 1930; from white fish eggs from the State Fish Hatchery at Bay City, Michigan, November 19, 1930; from a bullhead in the fish laboratory in the Division of Fisheries Research, University of Michigan, November 19, 1930; from a horned dace and from rainbow brook trout eggs from the United States Fish Hatchery at Northville, Michigan, January 23, 1931; and from rainbow trout eggs from the United States Fish Hatchery at Charlevoix, Michigan, March 5, 1931. Unless otherwise stated, all studies reported in this paper were made from cultures derived from single zoöspore isolations obtained from the white fish eggs. Although this collection was chosen arbitrarily as the one with which the special experiments were carried out, cultures made from single zoöspore isolations from each of the other sources were carried for comparative studies.

In addition to the single zoöspore cultures, infected fish, as well as gross cultures of white fish eggs and of rainbow trout eggs, both the original infections and those developed subsequently in the laboratory, were under observation throughout a period of several months. All cultures were kept in a refrigerator at a temperature of 10° C.

EXPERIMENTAL

The first experiments consisted of a series of cultures in which fish eggs were used as the substratum. The use of trout eggs

was abandoned almost at the start as their large size made them obviously unsuited to experiments involving the reduction of food supply. White fish eggs which are much smaller were used almost exclusively and even though they are more delicate and require more careful technique in keeping them alive in the laboratory, they were better suited to culture work. Living eggs, dead eggs and eggs that had been sterilized in the autoclave were used. The amounts of water and the number of eggs used were varied in an attempt to reduce the amount of food supply to such proportions that the formation of oögonia and antheridia might be produced on the natural substratum on which the fungus ordinarily grows. From one to six eggs were used in amounts of water varying from 15 c.c. to 500 c.c. In the cultures in which dead eggs were used either sterilized or unsterilized, without reference to the amount of water in which they were placed, the fungus grew luxuriantly and produced zoösporangia and an abundance of chlamydospores but no sexual organs. When living eggs were employed the situation became at once complicated. It involved the handling of a very delicate host that is extremely sensitive to laboratory treatments. White fish eggs die quickly kept in conditions that obtain in a laboratory, especially when the water contained in a culture in which they are used can not be aërated or renewed. Living eggs can be distinguished easily from the dead ones by their translucent or semi-transparent appearance. Dead eggs have at first a white chalky spot and later have a solid white look throughout. The difficulty is increased since it is impossible to do more than thoroughly and carefully wash the fish eggs in an endeavor to free them from any zoöspores from some outside source; consequently the possibility of contaminations is always present.

From one to three living eggs were removed to the culture dishes containing 15 c.c. of sterile distilled water and zoöspores. In all of the experiments in which the eggs died within 48 hours, infection took place on the dead eggs, but as far as could be determined not while they were alive. In the lesser number of instances in which eggs remained alive for more than 48 hours, no infection occurred even after the eggs died. One would infer from this that the zoöspores could not gain access to the living eggs

and that the death of the zoöspores took place before the death of the fish eggs. Even in the event that one of the three eggs remained alive longer than the others, the living one was not infected while the dead ones were immediately attacked. It was never until the telltale white spot appeared on the egg that the fungus seemed to have gained a foothold. If, however, mycelium instead of zoöspores was used as the source of infection, the dead eggs eventually all became infected. The reserves of protoplasm in the mycelium outlive the zoöspores and as new zoöspores are produced from these reserves throughout a relatively long time, it is assumed that some viable ones were present to infect the eggs as soon as they were dead.

A second series of experiments in which fish eggs were used was made in an endeavor to reduce the diffusion or availability of the nutritive supply. Sterilized white fish eggs were embedded in paraffine, using the technique described in a previous paper by the author (**5**). Single eggs were cut out with a shell of paraffine surrounding each. Some of the paraffine shells were punctured with a sterile needle while others were left sealed. One such egg was used in a capsule containing 20 c.c. of distilled water. Zoöspores were added to these. In the unpunctured ones no infection occurred. In the punctured ones there was a feeble growth of flaccid hyphae bearing at length atypical zoösporangia and chlamydospores. No sexual organs were seen.

Sterilized fly legs, small flies and plump house flies that are used so successfully as media for many species of *Saprolegnia* were tried as culture media for this species. The fly leg cultures were so feeble and the mycelium so impoverished that only a few sporangia and chlamydospores were produced and they were as atypical as those produced in the paraffined fish egg cultures (FIGS. 7 AND 15). In both of these a reduction was effected but it was so drastic that not even a good mycelium could be maintained. Klebs (**7**) pointed out that it is imperative that a well-nourished mycelium precede sexual reproduction, so that the negative results in these two instances could be predicted from the general macroscopic appearance of the mycelium.

Other fly cultures produced a rapid and luxuriant growth of mycelium with typical zoösporangia and countless chains of

chlamydospores. Many of the secondary zoösporangia were of the branching *Achlya* type instead of the usual proliferating *Saprolegnia* type. In the fly cultures the chlamydospores were the conspicuous feature even in young actively growing cultures and the fungus possessed all of the characteristics that it exhibited when fish or fish eggs were the media.

In another series of experiments hemp seeds were used. Two sterilized seeds were placed in 20 c.c. of sterile distilled water. Zoöspores obtained from the six collections of *S. parasitica* were tried. At the end of 4 weeks many sexual organs in the early stages of development were found in the culture that had been derived from the white fish eggs. The oöspores developed the characteristic heavy walls during the following days. After six weeks a few sexual organs were found also in the hemp seed culture derived from the trout eggs. The cultures from the other sources showed no oögonia and antheridia and this difference together with some others that can not be discussed here leads us to infer that there are undoubtedly physiological forms within the species.

While the foregoing experiments were in progress a series of cultures had been prepared to determine with more exactness, by the use of solutions, the kind and amounts of nutritive material that would favor sexual reproduction. A well-nourished mycelium was obtained by growing the fungus in pea broth for 5 days. It was then washed in sterile distilled water for 1 hour and small pieces of the mycelium of approximately the same size were used as the inocula. The materials found effective by Klebs (l.c.) and by Kauffman (l.c.) were selected as those to be used: haemoglobin, leucin, peptone, maltose, glucose, levulose, sucrose, $MgSO_4$, KH_2PO_4, $Ca(NO_3)_2$, K_3PO_4, K_2SO_4, KNO_3. The solutions were prepared in per cents .5 and .1 and were diluted with sterile distilled water to the lower per cents desired. They were not sterilized but were handled with a great amount of care. The culture dishes and the fungus were bacteria free. The cultures were quickly transferred to a refrigerator at 10° C. where they were kept except when examined.

The sugars and salts used were C. P. American manufacture, the haemoglobin and leucin were from pre-war German stock;

Witte's meat peptone was the peptone used in the regular experiments and unless otherwise stated is the one meant. However, other brands were used in comparison: Eimer and Amend, Chassing; Eimer and Amend meat peptone; Eimer and Amend peptone from albumen; Armour and Co. peptone siccum; Fairchild's peptone; Digestive Ferments Co. bacto-peptone; Parke Davis and Co. peptone.

In the experiments in which the salts were used alone, the mycelium was quickly impoverished and soon appeared to be dead. Even in low concentrations, .0125 per cent, they were uniformly detrimental.

In haemoglobin solutions the fungus made an excellent vegetative growth. Even in weak concentrations or with the addition of the inorganic salts the mycelium still grew well and formed many chlamydospores. The number of chlamydospores and their size was in direct proportion to the strength of the solution in which the fungus was growing. Many of them were solitary rather than in chains. They remained in a resting condition throughout several months.

The following per cents were used alone and in combination with varying amounts of one salt in the per cents indicated below.

Haemoglobin alone—.01, .025, .05, .00625, .0125 per cent.
Haemoglobin .0125 and $Ca(NO_3)_2$, $MgSO_4$, KH_2PO_4, .1 per cent.
Haemoglobin .05 and $Ca(NO_3)_2$, $MgSO_4$, KH_2PO_4, .05 per cent.
Haemoglobin .025 and $Ca(NO_3)_2$, $MgSO_4$, KH_2PO_4, .1 per cent.
Haemoglobin .025 and K_2SO_4, K_3PO_4, KNO_3, .1 per cent.
Haemoglobin .00625 and K_2SO_4, K_3PO_4, KNO_3, .05 per cent.

Leucin was found to be very effective also for the development of the vegetative stage and was more satisfactory than haemoglobin since it allowed a gradual, vigorous growth of mycelium. Large numbers of zoösporangia of the typical shape and size were produced, and tardily after about two weeks, chlamydospores were formed. They resembled oögonial initials in that they were terminal and were subspherical in shape. The culture looked very much like a culture of *Allomyces arbuscula* Butler with its resting spores. Solutions containing leucin in the

following concentrations were tried; .05, .025, .001, .0125, and .00625 per cents; leucin .025 per cent and each of the three salts KH_2PO_4, $Ca(NO_3)_2$ and $MgSO_4$ .1 per cent. All of these allowed excellent growth of mycelium and the production of zoösporangia and chlamydospores. Leucin .0125 and KNO_3, K_2SO_4, K_3PO_4 in any of these per cents used separately was unfavorable for even vegetative growth. The protoplasm was transformed into zoöspores and the fungus was exhausted.

The four sugars selected were tried both alone and in combination with the inorganic salts with the following results: Sucrose and maltose were used in concentrations separately .05, .025, .0125, .00625 per cents. Levulose and glucose were used in .1, .25, .025, .125, .2, .05, .625, .0125 and .5 per cents. An examination at the end of 8 days and again at the end of three weeks showed clearly that levulose, glucose and sucrose were ineffective for either good vegetative or reproductive growth of this fungus. The mycelium scarcely maintained itself and little if any new mycelium was formed. The additions of salts to these were not helpful. However, maltose was found to be decidedly effective in producing a fine vegetative growth. Normal zoösporangia were produced followed by chlamydospores. Mycelium made an excellent, gradual growth and appeared in many respects like that grown in the leucin solutions. The following combinations of maltose with the addition of a single salt were tried:

Maltose .1 per cent and K_2SO_4; K_3PO_4; KNO_3; .025 per cent.
Maltose .0125 per cent and K_2SO_4; K_3PO_4; KNO_3; .0125 per cent; .125 per cent and .00625 per cent.

In all of these the mycelium grew well and many chlamydospores that resembled oögonial initials were seen but no oöspores were produced in them.

Peptone was another source of nutrition that proved to be good for the vegetative growth and also to be a stimulus for the production of sexual organs when used with leucin glucose or maltose. Mycelium from the white fish egg isolation was grown in the eight brands of peptone in .05 per cent and in .025 per cent. There were some observable differences in the development of the

fungus in the different peptones but they were relatively insignificant, and at the end of ten days the mycelium in all of the experiments quite uniformly resembled the fungus as it appeared when it was grown on fish eggs. In siccum peptone the mycelium remained entirely vegetative longer than in any of the others, the amount of mycelium in Witte's peptone solutions was constantly less in amount, and in Chassing peptone the zoösporangia were unusually large and numerous. No sexual organs were developed in the solutions when the peptones were used alone. In certain combinations with leucin and with maltose many oögonia and antheridia were formed on the mycelium from the white fish isolation. Only the combinations of peptone with maltose and with leucin that were effective will be given. They were: Witte's peptone .025 per cent and leucin .025 per cent; Witte's peptone with maltose .025 per cent, and with maltose .0125 per cent, and with maltose .05 per cent; Witte's peptone .0125 per cent and maltose .0125 per cent; Parke Davis and Co. peptone .025 and maltose .025 per cents. In the two last named combinations the inoculum was derived from mycelium that had been kept in the laboratory for one year. The characters of the oögonia and antheridia in all of these were similar.

The question of the usability of various brands of peptone was tested in a number of experiments. One series was made using .05 per cent of each of the eight brands separately with .05 per cent of maltose. In a second series .025 per cent of each was used and in a third .0125 per cent. *S. parasitica* from the white fish eggs that had been kept in stock culture in the laboratory for one year and had been grown in fresh pea broth and thoroughly washed in the usual way, was used as the inoculum. At the end of ten days oögonia were observed in the mycelium in two of the solutions; Witte's peptone with maltose, .0125 per cents, and in the Parke Davis & Co. peptone with maltose .025 per cents. It would appear from the absence of sexual organs in the majority of these experiments, that there was either an excess or deficiency of some element or elements in the peptone that caused the variations in the reactions. The significant thing beyond the fact of differences in these brands of proteins is, of course, the fact that sexual organs appeared in

mycelium that had been grown under laboratory conditions for that length of time. It seems to preclude any notion that sexuality was merely the reaction to a forced condition that was brought about by the transference of the mycelium from fish substrata to the decidedly different environments maintained in the experiments. We can infer from this that sexuality is really an inherent capacity of the protoplasm. Witte's peptone used with haemoglobin was not effective as was found by Klebs (l. c.) and by Kauffman (l. c.) for "*S. mixta*."

The addition of salts to the maltose and peptone solutions inhibited the formation of sexual organs. Equal parts of the following per cents were used:

Maltose .025 + peptone .025 + K_3PO_4 .01
Maltose .0125 + peptone .0125 + K_3PO_4 .005
Maltose .05 + peptone .05 + K_3PO_4 .02
Maltose .0125 + peptone .125 + KNO_3 .0025
Maltose .025 + peptone .025 + KNO_3 .005
Maltose .05 + peptone .05 + KNO_3 .01
Maltose .05 + peptone .05 + K_2SO_4 .01
Maltose .0125 + peptone .0125 + K_2SO_4 .0025
Maltose .025 + peptone .025 + K_2SO_4 .005

Since maltose and peptone were found effective in liquid media, a synthetic agar medium was made in which salts were excluded excepting as they are contained in the peptone itself. Maltose .3 per cent, .6 per cent, 1.2 per cent together with Witte's peptone .1 per cent were used. An agar medium containing glucose 1.2 per cent and peptone .1 per cent was tried also. It is, of course, realized that the heat of sterilization brings about changes in the sugars and peptone so that the sources of nutrition are not comparable to those in the unsterilized solutions. On the glucose and peptone agar oögonia and antheridia formed in abundance within seven days. No sexual organs formed on the mycelium grown on the maltose .6 per cent and peptone, but a few appeared on the mycelium grown on the agars in which the .3 per cent and the 1.2 per cent maltose was used. In all there were ultimately produced a large number of spherical chlamydospores that resembled immature oögonia. The glucose and peptone

agar was found to be the most satisfactory medium for the development of sexual organs. The same good results were also obtained with mycelium that had been grown one year in stock culture in the laboratory.

DISCUSSION OF THE EXPERIMENTAL

It will now be necessary to analyze the foregoing results and try to understand some of the underlying principles that explain the apparently completely sterile condition of this fungus in nature and in most conditions of culture, and the several instances in which sexual organs were produced in culture. Starting with the Klebsian premise that an abundance of nutrition produces vegetative stages only, we find that that is exactly the reaction of this organism to the fish and fish egg substrata. It is so completely true for this fungus that for more than sixty years this fish parasite has been considered sterile. The superabundance of food gives rise to luxuriant mycelium and asexual reproduction. The tremendous numbers of chlamydospores are themselves a special type of reaction of the fungus to transform the almost unlimited food supply into protoplasmic reserves. Some of the environmental conditions established in the above experiments supplied a similar stimulus with the result that the vegetative and asexual dominated in these likewise. This was evident particularly in cultures using fish eggs, fish and flies, as well as in the haemoglobin and pea broth solutions.

In certain other experiments a condition was established in which a restricted vegetative growth was obtained and at the same time a vigorous, functioning mycelium was maintained. The maltose and the leucin solutions with peptone, the synthetic agar cultures with peptone and glucose, with peptone and maltose, as well as the hemp seed cultures represent this second class.

Still other experiments show the lower limits of adaptability as the fly leg, paraffined fish eggs substrata and the various inorganic salt solutions. Here the nutritive supply was so low that not even a good mycelium was produced.

With respect to the first group of experiments the situation is probably this; the nutrient was at once available in quantities,

and no proper check was found sufficient to cause control of the vegetative development and at the same time allow sexual reproduction. The fish and fish egg cultures all showed this condition and the haemoglobin solutions gave the same results.

When we consider the cultures that fall into the second group of experiments we find that there was a *gradual* growth of mycelium and concomitant with this reaction was the formation of a great number of zoösporangia and a small number of chlamydospores. There were formed also a large number of bodies that resembled oögonial initials. By modifying the maltose and leucin solutions by the addition of peptone the physiological environment was changed in some of the solutions to such an extent that sexuality was allowed expression. This is the explanation of the results of the leucin and peptone cultures, the maltose and peptone cultures and the glucose and peptone agar and maltose and peptone agar cultures. In Pieters' work (**8**) on *S. monoica* he found that a striking feature was "The very large number of oögonia found on the mycelium from .1 per cent and from .2 per cent peptone," while only one third as many were found on the mycelium grown in a solution of maltose and peptone. This is a very different reaction from that found in *S. parasitica*. As Pieters (**9**) points out, peptone is a complex of uncertain quantities of proteins and inorganic salts, so that in the final analysis it is impossible to ascertain the exact cause of the stimulus for sexuality. It is, obviously, impossible to analyze the conditions that took place in the hemp seed cultures. However, by inference it is partly explainable. The macroscopic appearance of the mycelium grown on hemp seed was sufficiently different from that of the mycelium grown on rich concentrations of the fish egg substrata to indicate radical differences in the physiological reactions. The mycelium on the hemp seed grew slowly forming a moderate amount of straight, shining hyphae although they were very different in appearance from the few flaccid hyphae produced in other slow growing cultures. Notwithstanding the lessened amount of mycelium it was in a vigorous growing condition and did not have at any time the appearance of a weakened plant. Pieters (l. c.) showed that well nourished mycelium is not necessarily coördinate with the

dry weight of the mycelium. This is inferred to be true, also of *S. parasitica*. The cultures of *S. parasitica* that represent the two extremes of good vegetative growth are the hemp seed culture and the fish egg culture. If vigor or well nourished mycelium was to be interpreted in terms of quantity, then the mycelium grown on the fish eggs should have been the mycelium on which the sexual organs appeared, but this was not the case.

The fungus grown on the hemp seeds seemed in many respects to appear more normal, judging by a comparison with what we interpret a well-balanced growth to mean in other species of the genus *Saprolegnia*. The zoösporangia and secondary zoösporangia were very regular in shape and size. The chlamydospores were never as numerous or as large as under certain other conditions and the sexual reproduction was present. The oögonia and antheridia appeared at the end of four weeks. That they appeared so late is undoubtedly a direct positive response to the decreasing amount of nutritive supply available. This is also evidenced by the fact that the amount of protoplasm in the hyphae was very greatly reduced. The hyphae immediately surrounding the seeds were nearly devoid of protoplasm, so that the mycelial weft had a semi-transparent appearance.

A comparison will now be made of the results described above with similar physiological work done by other investigators on fungi in this group. Klebs (**7**) and Kauffman (**6**) in working with "*S. mixta* de Bary" found that potassium phosphate was particularly effective in increasing the appearance of sexual characters in haemoglobin or leucin solutions. Kauffman also states that potassium nitrate, potassium sulphate or calcium nitrate with leucin, or potassium nitrate with haemoglobin were used successfully. For *S. hypogena* Pringsh. he also found potassium nitrate and potassium phosphate with haemoglobin to be a favorable stimulus. None of these salts were effective in *S. parasitica*. In working with *S. monoica* Pringsh. Pieters (l. c.) found that when a well nourished mycelium was placed in a .05 per cent haemoglobin solution that oögonia were readily formed but that not all of them had antheridia. He states further that "Levulose is used more readily (by *S. monoica*) than other sugars except maltose, and that it has a much greater effect in developing

a tendency toward production of oögonia than maltose has." He states that phosphates exert a marked effect upon the number of oögonia produced in this species. We have shown the use of levulose or phosphates as he describes were ineffectual with *S. parasitica*.

Pieters also studied the reactions of a variety *vexans* Pieters of *S. monoica* that he obtained from a dish of algae from a lake near Ann Arbor. In this species no sexual organs were formed in cultures maintained for a year and a half on flies, on agar, and in solutions of haemoglobin, leucin, peptone, etc. However, sexual organs were produced on the mycelium that was transferred to a solution containing leucin M/200 and levulose M/200. He says, "We seem to have here, therefore, the remarkable case of a variety of *S. monoica* having lost sexuality and recovering it under stimulus of this special combination. . . ."

For *S. ferax*, Coker (**8**) reports that in 5 per cent maltose and .01 per cent peptone used in equal parts, oögonia and antheridia were produced. He says, "This is the only *Saprolegnia* that forms normal oögonia and eggs in this medium."

The outstanding result of this brief comparison of results shows clearly that each species has a very different set of physiological reactions, but a critical analysis also shows that the underlying principle of reduced supply of nutritiment as a means of governing the appearance of sexual organs is applicable to all.

MORPHOLOGY

The most conspicuous feature of *S. parasitica* in microscopical mounts of mycelium made from infected fish is the countless numbers of dense chlamydospores. They form long chains that often measure 4 mm. in length, and contain often as many as twenty in a single chain. They are present in such numbers that they make the mycelium appear white, and individual chlamydospores can be seen easily with a hand lens. There are many variations in shape and size depending largely upon the substratum on which the fungus is growing. Some are nearly spherical (FIGS. 5 AND 12), others are long clavate or pyriform (FIGS. 4 AND 7), and still others resemble links of sausage. In some salt solutions they form abnormally in terminal bunches

(FIG. 13) and look not unlike zoösporangia of *Phytophthora* spp. Only slight diagnostic value can be attached to the number, size or shape of these bodies so that these variations can have no appreciable taxonomic significance. The chlamydospores are, however, always so numerous in the mycelium that they present a striking appearance and they undoubtedly will continue to be used as a clue to the determination of the fungus found on fish.

They separate easily from each other (FIG. 14) so that individuals are successfully disseminated. Huxley (**4**) mentioned this characteristic as conspicuous in the fungus on salmon in England. They regenerate either by sending out stout hyphae or by being transformed into zoöspores. Repeated changes of water will bring about the formation of tremendous numbers of zoöspores. They even form in the droplet of water surrounding a chlamydospore transferred to agar plates.

Another feature concerning the chlamydospores is the formation of tube-like processes through which the zoöspores are liberated. A single terminal chlamydospore just as frequently has several of these tubes as do the chlamydospores in the middle of a long chain (FIGS. 3, 4 AND 8). These tubes, of course, are not homologous with the exit tubes of other oömycetes. The tubes or projections are filled with protoplasm which may be transformed into zoöspores (FIG. 9). Further growth by means of hyphae may take place. The solitary spherical or pyriform chlamydospores strongly suggest oögonial initials. Indeed it is evident that they may remain as chlamydospores, function as zoösporangia or as oögonia with or without antheridia, depending upon conditions of the environment. The empty chains of chlamydospores with their tubes often give the appearance of an old culture of *Allomyces arbuscula* Butler with its chains of empty zoösporangia.

The typical zoösporangia are long cylindrical (FIG. 1). They measure 180–350+ × 20–24 μ. There may be a great variation from this shape and size. The first ones formed are very unlike the small irregular sporangia that appear in old mycelium or on mycelium growing in unfavorable conditions, such as are represented by the fly leg cultures (FIGS. 7 AND 15). The secondary

zoösporangia are normally proliferating (FIG. 20), but under some conditions they form as in the genus *Achlya* (FIG. 4). Coker (2) mentions this in his description. The zoöspores are diaplanetic (FIGS. 17 AND 18). They measure 12 μ in diameter.

Oögonia and antheridia were observed in four types of cultures discussed previously in this paper. The characters of the sexual organs produced on the solid medium and in the liquid media were similar in most respects. The shape of the oögonia and the place of origin of the antheridial branches were the chief differences. On the solid medium nearly all of the oögonia were spherical and the antheridial filaments were androgynous (FIGS. 27 AND 28). Many oögonia developed without antheridia (FIGS. 29 AND 31). The oögonial walls were sometimes thickened, never pitted, and the basal wall frequently projected convexly far into the oögonial cavity (FIG. 30). The last named feature seemed to be an abnormal reaction. In the liquid environments the oögonia were spherical, subspherical to pyriform or clavate, and many were intercalary. The antheridial branches were always diclinous in origin and were very long, slender, winding filaments (FIGS. 22, 23 AND 26). Very few oögonia developed parthenogenetically.

On the mycelium grown on the hemp seeds, the oögonia were either terminal or intercalary (FIGS. 23 AND 26). The walls were very thin, smooth and unpitted. The antheridial branches were diclinous in origin. The spherical oögonia measured 65–95 μ in diameter. The pyriform ones reached 135 μ in length.

The oöspores number from three to twenty-five. They are subcentric and measure 18–22 μ (FIG. 32). They take on a rich golden-brown color even before the oöspore walls are completed. They usually fill the oögonium though not always. The parthenogenetically formed oöspores were scarce in the hemp seed cultures, frequent in the leucin and peptone solutions, and were abundant on the mycelium grown on the agar media.

The antheridia were always small clavate or pyriform organs. The number on an oögonium varied in the different media. There were seldom more than one antheridium attached to each oögonium in the maltose-peptone, or leucin-peptone solutions, but there were commonly from two to four on each oögonium on the mycelium grown on the hemp seed, while on the agar

medium only one was found. Many suboögonial branches had every appearance of being the beginnings of antheridial branches, but often they developed as slender hyphae instead (FIG. 30). The passage of the contents of an antheridium into an oögonium was watched in one instance.

TECHNICAL DESCRIPTION

Saprolegnia parasitica Coker (Emend.) Saprolegniaceae, *Pl. 18*. 1923.

Vegetative plant filamentous, mycelium forming a dense weft. Chlamydospores abundant, usually in chains of from 3 to 30 in a row; in certain culture media terminal and solitary resembling oögonial initials; variable in shape, long cylindrical, clavate, pyriform or subspherical to spherical; separating easily, regenerating by means of hyphae or by the formation of zoöspores; size variable, tube-like processes common.

Asexual reproduction by means of zoöspores. Zoösporangia regular, long cylindrical, 180–350 × 20–24 μ, smaller and irregular in shape in old mycelium or in certain culture media. Secondary zoösporangia proliferating, sometimes forming as in the genus *Achlya*. Zoöspores diaplanetic though in unfavorable conditions germinating in situ. 12 μ in diameter.

Sexual reproduction by means of oögonia and antheridia. Oögonia terminal or intercalary; pyriform, clavate, subspherical to spherical in shape; 65–135 × 60–75 μ or 65–95 μ in diameter. Walls very thin, colorless, smooth, unpitted. Oöspores variable in number 3–25 (35) usually filling the oögonium; subcentric; colored rich golden-brown. Parthenogenetically formed oöspores frequent in some conditions of culture. 18–22 μ in diameter. Antheridial branches diclinous or androgynous in origin, the latter long, slender, winding filaments. Antheridia small clavate to subcylindric; 1 to 5 attached to each oögonium.

Habitat: parasitic and saprophytic on various species of fish and on fish eggs. Common in Michigan and reported from various parts of the United States and elsewhere.

PARASITISM

The whole problem of parasitism of fish and fish eggs is a complex one. In connection with the experiments described

above in which living fish eggs were employed brief comments were made on the difficulty attending the use of eggs as media, since any process of sterilization is ruled out. The same difficulties arise with the use of living fish or fry. The foundations upon which experiments requiring these media must rest are always questionable since there is always the danger of the presence of contamination from spores from some unknown source. In connection with the physiological studies reported herein, a few interesting facts came to light. They are so meager that but little significance can be attached to them, yet they do offer some interesting data.

Some of the trout eggs that were brought into the laboratory November 19, 1930, hatched, and the fry were kept alive in a healthy condition and were to all appearances entirely free from fungus infection. In March, 1931, three of them were isolated for experimentation. One was put into each of three small covered capsule culture dishes, containing 20 c.c. of sterile distilled water. One of the three was killed, one was slightly injured and the third was left unharmed.[3] The dead fry and the injured one were attacked immediately and within three days they were surrounded with a dense weft of mycelium. The uninjured fry continued to live in the same healthy condition for two weeks. At that time more zoöspores were added to the water. Infection took place after this last procedure and the fry was killed within two weeks. The hyphae appeared around the mouth parts first. In the last analysis it cannot be said that infection took place here on sound tissue as it is well known that fish are easily injured by continually hitting against the glass walls of aquaria. It is not unlikely that that is what happened here, although it is just as reasonable to suppose that the resistance had been lowered by the extraordinarily poor conditions under which the fish was compelled to live and that it became infected in a weakened condition. The remarkable fact remains that the fry withstood the first onslaught of infection at all.

There are also the interesting questions concerning the infection of living eggs by fungus hyphae. Can tips of growing hyphae penetrate living eggs, or is infection restricted to the germ tube hyphae from zoöspores adhering to the surface of the eggs?

[3] Zoöspores were put into each of the three capsules.

Does infection take place actually before the eggs die, at the time of death or immediately after they die? Observations made on masses of eggs that are brought into the laboratory from the hatcheries indicate that penetration does take place directly into the living eggs by hyphae of vigorously growing mycelium. A single infected egg can form a center of infection and rapidly the eggs surrounding this one will be killed and fastened together by the mycelium into tight knots. Undoubtedly the pressure of growing hyphae on the egg membranes when the eggs are held so tightly together is sufficient to allow penetration of the mycelium into living eggs. It seems rather doubtful that zoöspore infection could cause such rapid invasion as does occur. Infection becomes so disastrous in bulks of eggs within an incredibly short time that it is often impossible to save many of the eggs. Loss of the eggs cannot, however, be said to be exclusively due to fungus infection, as it is well known that large numbers of eggs die because of unfavorable physical conditions alone.

The question seemed to take on a different aspect in the laboratory where only about a dozen or twenty eggs were contained in one-half liter of water. In this situation the pressure of the hyphae on isolated eggs is minimized and it is unlikely that here the fungus can gain entrance into living eggs. Repeated observations showed that living trout eggs that were lying near dead, badly infected ones remained alive for a considerable length of time. Even when infection did take place it was more than probable that it was due to the germ tube of some zoöspore that adhered to the egg, and that infection took place at or near the time of the death of the egg. This resistance of eggs seems to suggest that the entrance by penetration of hyphae must be preceded by some radical change in the egg membranes, enzymatic, toxic or otherwise that accounts for the parasitic reaction of the fungus. A mere loss of vitality in the egg may presumably lower the resistance to a point where the fungus can gain access.

Infected fish are common in aquaria and in lakes. The question of whether they are attacked when they are in a healthy condition and whether death is due to the invasion of the fungus

is a difficult one to study. Huxley (**3** and **4**) made a careful and detailed study of the epidemics that were occurring in fish in Scottish and British rivers and concluded that healthy fish could be attacked. He stated, further, that upon the return of the salmon to the salt water the fungus was not troublesome. The extermination of the fungus in hatcheries and in the fresh water lakes and streams is, of course, utterly impossible. The only hope in the hatcheries is in using unremitting care in promptly removing infected fish and eggs and in making proper disposal of such material. The established practice in hatcheries of continually removing the infected eggs as soon as they are discovered and also in removing all dead eggs from the trays is of utmost importance. All infected material should be disposed of in such a way that it cannot continue to contaminate the source of water in which fish are being reared. It is scarcely possible to over-emphasize the importance of these two points wherever the propagation of fish is concerned.

SUMMARY

1. Isolations of *Saprolegnia parasitica* were made from five localities in Michigan and were taken from five species of fish.

2. Detailed physiological experiments were made on the mycelium derived from a single spore isolation from white fish eggs obtained from the State Fish Hatchery at Bay City, Michigan. The experiments were made to determine if possible whether or not *S. parasitica* was a sterile species as has been supposed.

3. It has been shown that the morphology of this fungus can be controlled largely by modifications of the environmental conditions. These modifications are based upon the Klebsian hypothesis that vegetative and reproductive processes can be controlled by a gradual reduction of the nutritive supply.

4. Oögonia and antheridia were produced on the mycelium in certain experiments tried. This is the first time that sexual organs have been shown to exist for *S. parasitica*.

5. Peptone was found to be the stimulus for sexual reproduction when used in combination with leucin or with maltose in solution, or with glucose or maltose in synthetic agar. Sexual organs were also produced on mycelium grown on sterilized hemp seed in water.

6. The morphological characters are unlike those of other species of the genus and support is thereby given for considering *S. parasitica* a distinct species.

7. The physiological reactions of *S. parasitica* are unlike those reported for related species and the doctrine of specificity is again confirmed.

8. The reactions of the fungus to substrata in nature are discussed. Given a highly concentrated nutritive supply like fish or fish eggs, *S. parasitica* produces an excellent vegetative growth and reproduces only asexually. Countless chlamydospores are produced as the reaction to a superabundance of food materials. Physiological experiments are reported in which the conditions found in nature are approximated. Conditions causing significant differences in physiological reactions are given in detail.

9. A few observations on parasitism are reported.

10. An emended description of the species is given.

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EXPLANATION OF PLATES

Saprolegnia parasitica

PLATE 12

Figs. 1 and 2. Empty zoösporangium with beginning of secondary proliferating zoösporangia. Typical cylindrical shape of the kind predominating in hemp seed cultures, and in certain concentrations of leucin, haemoglobin, and maltose.

Figs. 3, 4, 5, 6, and 7. Chlamydospores formed terminally, showing tubes typical of those seen in many cultures.

Figs. 8, 9, 10, and 11. Chains of chlamydospores formed on fish eggs, fish, flies, etc. Many chains are longer than these.

Fig. 12. Solitary chlamydospores resembling in size and shape the resting spores of *Allomyces arbuscula;* common in haemoglobin solutions.

Fig. 13. A group of chlamydospores showing the atypically clustered arrangement found when grown in solution containing K_3PO_4.

Fig. 14. Two chlamydospores becoming separated from each other.

Fig. 15. Empty chlamydospores and regenerating hyphae such as form the *Achlya*-like secondary zoösporangia.

Fig. 16. Two chlamydospores, the lower one functioning as a zoösporangium.

Fig. 17. Zoöspores in first swimming state (stained with potassium iodide).

Fig. 18. Zoöspores in second swimming stage.

Fig. 19. Germinating zoöspores.

Fig. 20. Proliferating secondary zoösporangia, formed in .025 per cent maltose solution.

PLATE 13

Fig. 21. Oögonium with antheridium of androgynous origin from mycelium grown on glucose and peptone agar.

Figs. 22, 23, and 24. Oögonia with antheridia of diclinous origin from mycelium grown on sterilized hemp seed.

Fig. 25. Oögonium with parthenogenetically formed oöspores. This undoubtedly represents a chlamydospore that has developed into an oögonium.

Fig. 26. An intercalary oögonium. Note the several antheridia attached from mycelium grown on hemp seed.

Figs. 27 and 28. Young oögonia with androgynous antheridial branches, from mycelium grown on glucose and peptone agar.

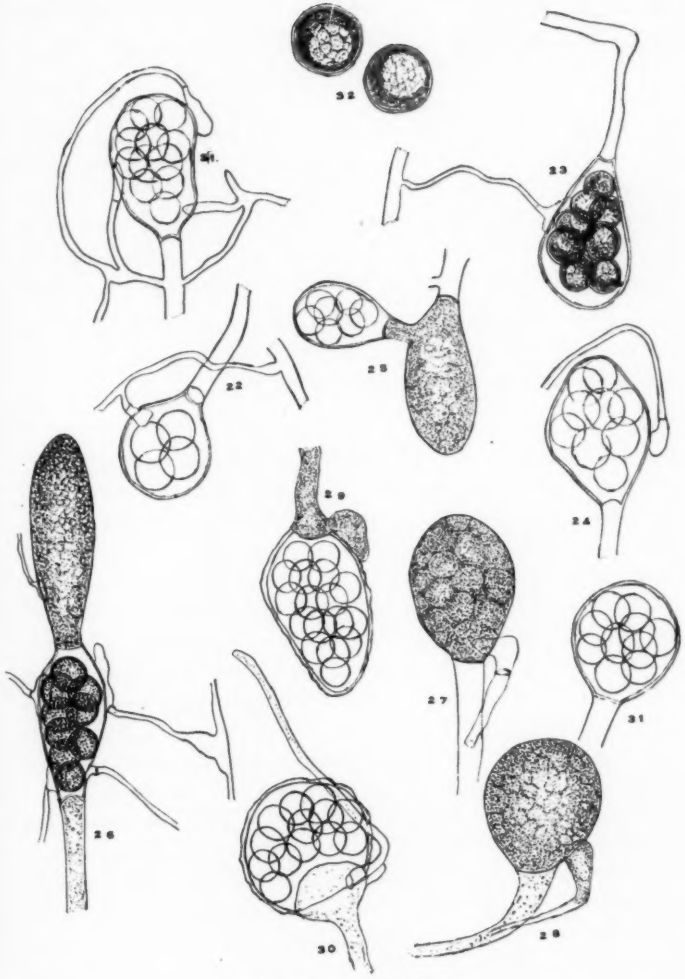
Fig. 29. Oögonium with parthenogenetically formed oöspores. The bulbous branch at the base is not an antheridial branch. If it were, it would be empty before spores were in the advanced stage of development represented n this figure. Many such branches are found in mycelium grown on the synthetic agar medium.

Fig. 30. An oögonium formed on mycelium grown on glucose and peptone agar. Note the thick roughened wall, the basal wall which projects far into the oögonial cavity and the branch from the oögonial filament.

Fig. 31. A small spherical oögonium with parthenogenetically formed oöspores, grown on synthetic agar medium.

Fig. 32. Mature, subcentric oöspores.

SAPROLEGNIA PARASITICA



SAPROLEGNIA PARASITICA

PSEUDOPYTHIUM PHYTOPHTHORON A SYNONYM OF PHYTOPHTHORA CINNAMOMI [1]

F. P. MEHRLICH

The genus *Pseudopythium*, represented by the single species *Pseudopythium phytophthoron* has been mentioned by Sideris (**4**) and by Sideris and Paxton (**2**, **3**) as a new genus in the family Pythiaceae, and type cultures have been distributed. A formal description prepared by Sideris was accepted for publication in MYCOLOGIA, and was cited by him as follows:

> "Sideris, C. P. Taxonomic studies in the family Pythiaceae III. *Pseudopythium phytophthoron* gen. et sp. nov., a parasite of pineapple plants. Mycologia **23**: in Press, 1931."

That author states in his manuscript: "It (this organism) was first considered as a species of *Phytophthora*, but after more careful study it was found that it differed from the true *Phytophthora* type of organisms. The true position of *Pseudopythium* is not well known. The organism can be placed neither in *Pythium* nor in *Phytophthora*, because it has failed, so far, to produce zoösporangia. The morphological characters, however, on which its differentiation and segregation from the other two genera have been based is of such prominence as to justify the creation of a new subgenus in either *Pythium* or *Phytophthora* in case its zoösporangial stage becomes known."

The writer of the present note, in studying the diseases of pineapple with which this organism is associated, has obtained the zoösporangial stage not observed by Sideris. Swollen hyphal vesicles which Sideris termed conidia function as chlamydospores only. The morphology of these vesicles, the proliferation of the zoösporangia, as well as the size and shape of the mycelium, conidia, and zoöspores all agree favorably with the description of

[1] Published with the approval of the Director as Technical Paper No. 36 of the Experiment Station of the Association of Hawaiian Pineapple Canners, University of Hawaii.

Phytophthora Cinnamomi Rands (1). Moreover, repeated studies demonstrate that the pathogenicity of this fungus agrees with that given by Tucker (5) for *P. Cinnamomi* Rands.

Subcultures of two isolations of this organism, which were isolated from separate areas on the island of Oahu, were sent to Drs. L. H. Leonian and C. M. Tucker under the tentative name of *P. Cinnamomi*. Each of these investigators independently verified the designation.

Because of these facts the description of *Pseudopythium phytophthoron* has been withdrawn by Sideris and will not be published. The name thus stands without description as a synonym of *Phytophthora Cinnamomi* Rands. In this light the contributions of Sideris and Paxton, cited below, include additions to the knowledge of the pathogenicity and geographic distribution of this species of *Phytophthora*, regarding which little has been written.

A later paper will consider in detail the morphological and physiological characters which establish the synonomy.

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A NEW FUNGUS FOR THE UNITED STATES

LEROY DONALD

While out collecting plants for taxonomic purposes in May, 1930, conspicuous galls were noticed at or near the surface of the ground on the stems and leaves of *Veronica arvensis*, the plant commonly known as Corn or Wall Speedwell. Thinking that perhaps this was nematodic injury, some of the material was carried into the laboratory for examination. The examination revealed the presence of a fungus instead which was identified as *Sorosphaera Veronicae* Schr.

Reference is made to the genus *Sorosphaera* in Engler and Prantl's Die naturlichen Pflanzenfamilen, I. Teil, Abteilung 1, page 7. Schröter in 1886 listed it as one of four genera under the Phytomyxinae, and made it to include the species *Sorosphaera Veronicae*. In his discussion, three host plants were described from Germany, namely *Veronica hederifolia*, *Veronica triphylla*, and *Veronica Chamaedrys*. In 1895, Rostrup found this species occurring on *Veronica hederifolia* in Denmark. Trotter in 1904 reported the organism in *Veronica arvensis* from Italy. Maire and Tison in 1909 and Blomfield and Schwartz in 1910 conducted a further and more detailed study of the organism, the embodiments of which were published during those years in Annales Mycologici and Annals of Botany, respectively. In 1929, Cook and Schwartz, while conducting an investigation of the root fungi in ponds and marshes around London, found and described from roots of a number of different grasses growing there a second species of the genus *Sorosphaera* which they named *Sorosphaera radicalis*.

The work on this genus mentioned thus far has been conducted in countries other than the United States, however, and as far as the writer has been able to ascertain, the presence of either species on any host has never been recorded in this country.

As stated by Schröter and corroborated by Blomfield and Schwartz, *Sorosphaera Veronicae* is parasitic in the parenchy-

matous cells of the living plants, causing swellings or tumours. In accordance with the description given by the last mentioned workers, the tumours may be found in various parts of the plant, their most common form being that of swollen stunted stems from which spring a few small deformed leaves. A given plant may have tumours in various stages of development; stems, petioles, and leaves may all show the presence of the fungus. Within the enlarged parenchyma cells of the tumours or swellings may be found the spores which are wedge-like in shape, and united in large numbers within a common membrane to form a spherical hollow ball, varying in size from 15 to 20 microns. Frequently the spore balls or sorospheres may become fused together or united, forming oblong or ellipsoidal compound structures. The tumours or swellings, shortly after the spores have been produced, turn brown and eventually rot, liberating the enclosed sorospheres.

Additional cytological work is being carried forward by the writer with the idea in mind of publishing at a later date a complete and detailed account of his investigations.

BOTANY DEPARTMENT,
STATE COLLEGE,
MISSISSIPPI.

ON A NEW DAMPING-OFF DISEASE OF TEXAS BLUEBONNETS [1]

J. J. Taubenhaus and Walter N. Ezekiel

(With 1 Text Figure)

The Texas bluebonnet (*Lupinus texensis* Hook.) is a native annual plant and the official State flower of Texas. The plant grows in wooded areas, open prairies, and along railroad banks; and seems to thrive well in calcareous, neutral, and even slightly acid soils. The bluebonnet flower is showy, and usually lasts a long time on the plants or when placed in water, and is therefore highly prized as a cut flower. Under field conditions, the plants are very prolific and reseed themselves every year. The seeds usually begin to sprout around January; the plants grow rapidly and are usually in full bloom by March. Bluebonnet plants are very easy to transplant, provided this is done during the month of February, before the plants begin to show flower buds.

Early in January, 1931, Mr. P. B. Monosmith, in charge of the Texas Agricultural and Mechanical College greenhouses, brought in large numbers of plants, which were potted in five-inch pots and placed in a cool house at a temperature of 65° to 70° F. These plants started out well, but before long many of the plants began to lose their normal green color, wilt, and die, and in about ten days the majority of the plants were dead.

Microscopic examination of the crowns and roots of these plants showed a typical damping-off and an abundance of *Rhizoctonia* and *Pythium* hyphae in the decayed tissues. A number of petri-plate cultures were made from infected crowns and roots, and both the *Rhizoctonia* and the *Pythium* were recovered from most of the plants. Microscopic examination of the *Pythium* showed that it resembled closely *P. Debaryanum* Hesse. The *Rhizoctonia* did not produce basidiospores in culture, but its cultural characteristics resembled *Rhizoctonia Solani* Kühn

[1] Published with the approval of the Director as contribution No. 200, Technical Series, of the Texas Agricultural Experiment Station.

as isolated many times by the writers from potatoes, sore shin of cotton, and various infected ornamental plants.

These two organisms were grown on oatmeal agar, and used to inoculate healthy plants in sterilized soil. One hundred five-inch pots were filled with Lufkin fine sandy loam soil material as originally used by Mr. Monosmith, and then steam sterilized for two hours at 15 pounds pressure. These pots were then planted with healthy bluebonnet plants secured from a railroad bank at College Station, Texas. Before planting, the roots and crowns of each plant were washed several times in running tap water and carefully examined for possible infection or lesions, none of which were found. These plants were then quickly dipped in a 1 : 2000 solution of bichloride of mercury and planted in the sterilized soil in the pots, two plants per pot. The potted plants were then placed in the College greenhouse in a cool house. After the plants were well established, twenty-five of the pots were inoculated with the *Rhizoctonia*, twenty-five with the *Pythium*, twenty-five with a mixture of both *Rhizoctonia* and *Pythium*, and twenty-five left as checks. Approximately fifteen grams of the respective oatmeal-agar cultures were mixed with two inches of the moist surface soil of each pot.

The results of these inoculations are shown (FIG. 1 AND TABLE 1). *Rhizoctonia* and *Pythium* used separately or in combination

TABLE 1

INOCULATION OF POTTED BLUEBONNET PLANTS WITH PURE CULTURES OF *Rhizoctonia* AND *Pythium*. (Plants set out in pots January 18, 1931; inoculated February 20; final results March 1, 1931.)

Organism used	No. plants wilting of series of 50	Per cent infection
None (Checks)	0	0
Rhizoctonia	43	86
Pythium	49	98
Mixture of *Rhizoctonia* and *Pythium*	50	100

caused damping-off, while in every case the checks remained normal. Since all the uninoculated, check plants grown in the steam sterilized soil remained normal throughout the experiment, it appears probable that the original occurrence of the disease was from soil infestation rather than from previous infection of

the young bluebonnet plants. By care in the selection of the plants, and soil sterilization with steam, damping-off of Texas bluebonnet plants grown under indoor conditions can apparently be prevented.

FIG. 1. Damping-off of Texas bluebonnets, *a* check, uninoculated; *b*, inoculated with *Rhizoctonia; c*, inoculated with *Pythium;* and *d*, inoculated with a combination of both *Rhizoctonia* and *Pythium*.

SUMMARY

A new crown-rot and damping-off disease was found on Texas bluebonnet plants transplanted into the greenhouse. The disease was shown to be caused by *Pythium Debaryanum* and a species of *Rhizoctonia*. Both of these organisms were found to be soil-borne, and readily controlled by steam sterilization of the infested soil and disinfection of the young seedlings when transplanting.

COLLEGE STATION,
TEXAS.

TWO NEW SPECIES OF LACTARIA

GERTRUDE S. BURLINGHAM

(WITH 3 TEXT FIGURES)

Under the date of December 15, 1909, Prof. George F. Atkinson of Cornell University sent me a specimen of *Lactaria* regarding which he wrote "I am enclosing a specimen of *Lactarius* which I collected in 1904 at Smithtown, L. I., not far from Port Jefferson. Dr. Peck was with me at the time. We both thought it an undescribed species and I have it in my manuscript as *L. villozonatus*. I should be glad to have your opinion of it. You may keep the specimen." He also enclosed full descriptive notes.

Again sometime shortly after 1917 (I have not the date) Prof. Atkinson sent me together with full notes another *Lactaria* which he had named in his manuscript *Lactarius nigroviolascens*. This last summer during the series of mycological forays held at Ithaca in honor of the visit of the Danish Botanist Dr. Jakob E. Lange, some specimens of this same species were collected in a ravine at Mud Creek.

Since Professor Atkinson evidently had never published the description of either of these species of *Lactaria*, it seemed advisable that this should be done, and Dr. H. M. Fitzpatrick, Professor of Mycology, who has charge of the Atkinson Herbarium at Cornell University, suggested that I should arrange for the publication of Professor Atkinson's notes on these two species. He kindly gave me access to the herbarium and has furnished prints from Professor Atkinson's photographs for reproduction in this article.

Lactaria villozonata Atkinson, sp. nov. (as *Lactarius* in the manuscript).

"Pileus depressed, subinfundibuliform, margin first strongly inrolled, villose, pale ochraceous buff to cream buff or whitish, zoned, margin in age usually prominently crenate. Gills narrow subdistant, white, emarginate and adnexed, slowly stained brown where broken. Stem white, smooth, usually tapering downward, sometimes even. Milk watery, taste slowly acrid. Spores white.

Pileo e depresso subinfundibuliformi, pallido-ochracea-isabellina subalbicante, zonata, tempore udo viscida, margine primo involuto villoso demum crenato; lamellis angustis subdistantibus adnexis, subaequalibus simplicibus, albidis jam tactu tarde fulvis; stipite laevi, deorsum attenuato, albo fulvomaculato; lacte aquoso tarde acri; sporae albae.

"Plants scattered or tufted. Pileus 8–12 cm. broad, stem 2–4 cm. long, 1.5–2 cm. thick."

FIG. 1. *Lactaria villozonata.*

Herbarium number *20215*. Photograph Roll 1, No. 36.

The dried specimens show a few characteristics not mentioned in this description. The context is not very firm, and from adhering leaves it is plain that the pileus is viscid when moist at least. The lamellae are unequal and simple. The villose condition of the margin is similar to that of *Lactaria resima*. There are fulvous spots of various sizes and shapes on the stipe. Unfortunately the spore description had to be made from spores taken from the lamellae of the dried type specimens instead of from fresh material. The average size is 6.25 $\mu \times 7.5\ \mu$. There are scattered small tubercles on the surface, and when stained with iodine there are some connecting lines.

Lactaria nigroviolascens Atkinson, sp. nov. (as *Lactarius* in the manuscript).

"Pileus at first blackish brown (bistre), then umber and tawny olive as it expands, usually remaining darker in the center, pruinose, more or less rugose, margin at first incurved, in age expanded, the pileus becoming depressed, usually with an umbo, margin at length with short distant striae and crenate,

surface of pileus formed of upright, crowded, slender acuminate cells or hyphae. Gills adnate, decurrent in short lines, subdistant, white becoming ochraceous, in age usually darker. Stem even, colored like the pileus, minutely and densely tomentulose.

Pileo fuliginoso-umbrino, pruinato subrugoso, margine demum striato, stipite subconcolore; lamellis adnato-decurrentibus, subdistantbus, ex albido ochraceis; carne albo, fracta e lacte nigroviolacea; lacte copioso albo non acri; sporae ochroleucae; *L. ligniotae* propinqua sed carne fracta nigroviolascente certe distincta.

"Plants scattered, 5–6 cm. high, pileus 3–5 cm. broad, stems 4–6 mm. stout."

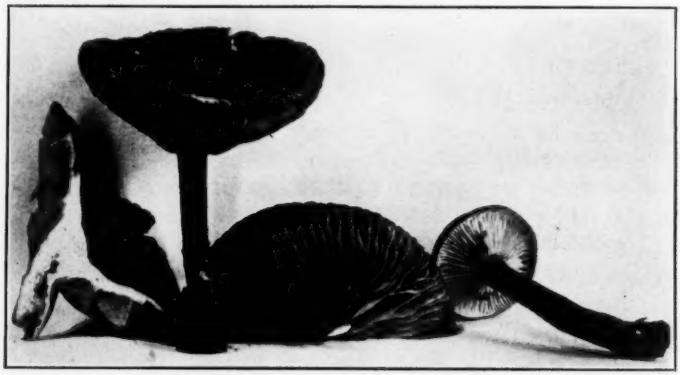


FIG. 2. *Lactaria nigroviolascens.*

"Flesh of stem and pileus white, milk white, abundant, slowly changing to blackish violet as do all wounds of the pileus, stem or gills when milk is present. As the plants become old and dry the milk is less abundant and only portions of the flesh change bright black on the pileus or stem. When the plant is fresh the change is to blackish violet. Spores echinulate, globose, with a large oil drop 8–10 μ. The species belongs in the Plinthogalae section of the genus."

Type locality: Ground, woods, Coy Glen, July 23, 1917. Herbarium number *24257*.

The following additional notes are from specimens collected on August 28, 1931 at Mud Creek near Ithaca.

Latex white, abundant, pungent but not acrid, staining the flesh dusky blue violet to plum violet (Ridgway). Spores ochroleucous t-1. Pileus mummy brown (Ridgway) to umber on the margin. Stipe chocolate t-1 to 2. Spores globose echinulate 10 μ in diameter inclusive of spines. When stained with iodine fine lines connecting some of the spines appear. I did not find an oil drop present.

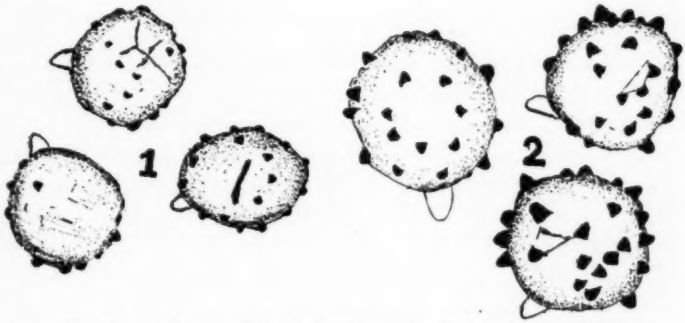


FIG. 3. 1, Spores of *Lactaria villozonata;* 2, Spores of *Lactaria nigroviolascens.*

This species closely resembles *Lactaria ligniota* Fries and may easily be mistaken for it unless one notes the difference in the color change of wounds. I did not find that the color of the latex changed except where in contact with the flesh.

I do not know what color book Professor Atkinson used in describing the color in his notes. In my additional notes I refer to the Repertoire de Couleurs except where otherwise indicated.

The type specimens of both of these species are filed in the Atkinson Herbarium at Cornell University, and one specimen from each is filed in my own herbarium.

The spore drawings have been made with the aid of a camera lucida using a 1/12 in. oil immersion lens and a 15 mm. ocular. The spores were stained with an iodine solution made according to the formula recommended by Richard Crawshay in "Spore Ornamentation of the Russulas."

BROOKLYN, NEW YORK

NOTES ON SARCOSPHAERA FUNERATA

ERDMAN WEST

(WITH PLATE 14)

According to Cooke[1] the type collection of *Sarcosphaera funerata* (Cooke) Seaver was made at Gainesville, Florida, by H. W. Ravenel. Seaver[2] stated that he had seen collections from New Smyrna, Florida; Albion, Michigan; and Melbourne, Australia (?). There seems to be no record that the plant had been collected in the original locality since the type collection was made in 1878. The writer collected several dozen plants along an old road near Gainesville during January and February in 1931, on sandy land that had not been cultivated for at least ten years. The vegetation of the area consisted of wire-grass (*Aristida stricta*) and cactus (*Opuntia* sp.) with plenty of bare ground between the plants. No specimens have been found in this area in 1932, probably because the field had been plowed and a house built on part of it.

In the descriptions given by Cooke and Seaver, there was no reference to a stipe on the apothecium. The right hand specimen on the plate published with Dr. Seaver's article apparently had a short stalk, but this fact was not mentioned in his article. Every specimen of this fungus so far found by the writer has had a stipe, but it was so brittle that it broke off unless the plant was dug with great care. On large plants, this stipe was as much as six centimeters in length and one in thickness, and it was proportionately less on smaller plants. Usually it was slightly longer than the diameter of the cup. It was concolorous with the outer surface of the apothecium and, like it, covered with adhering sand. The lower portion consisted of mycelium with a large proportion of sand. Although it was not mentioned

[1] Cooke, M. C. Ravenel's American Fungi. Grevillea **6**: 129–146. 1878.

[2] Seaver, F. J. Photographs and Descriptions of Cup-Fungi—XIII—Subhypogeous Forms. Mycologia **22**: 215–218. 1930.

in previous descriptions, many of the larger apothecia were plicate towards the base (PLATE 14, FIG. B).

FLORIDA AGRICULTURAL EXPERIMENT STATION,
GAINESVILLE, FLORIDA

EXPLANATION OF PLATE 14

Sarcosphaera funerata—Figure *A*.—Top view of five apothecia showing the typical star-shaped openings, which alone are visible when the plant is growing in its natural habitat. Figure *B*.—Lateral view of a plant showing the sand-covered stipe and the creases in the lower part of the cup. Figure *C*. —Another plant showing the stipe. Half of the cup has been removed to show the hymenium. (All photographs slightly enlarged.)

EDITOR'S NOTE

The above paper is a valuable contribution to our knowledge of this species (*Sarcosphaera funerata*). It is an interesting coincidence that just two weeks before this paper was received for publication the writer received from Dr. Lee Bonar, of the University of California, an interesting collection (obtained by one of his students) of the same species for determination with the following notes:

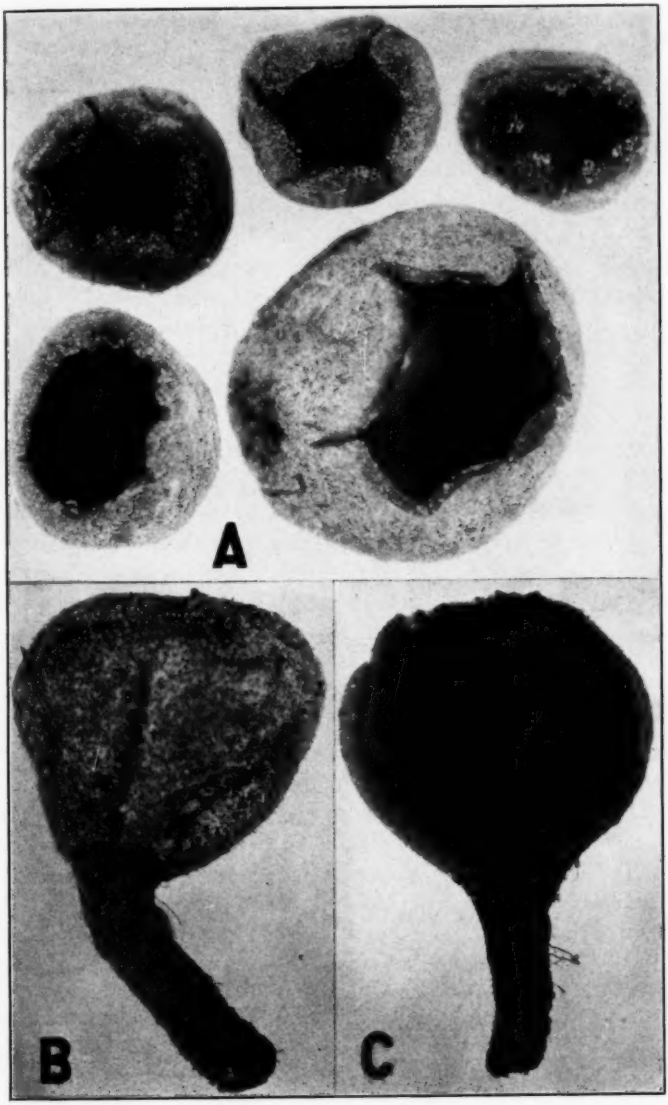
"The younger specimens are almost closed at the top, having a small hole, while the more mature ones are more opened, without any evident splitting down the sides. Exterior, simply the color of the sand which is held tightly to the exterior, in a mesh of whitish wooly hairs. Texture of the cup brittle fleshy, whitish in the sterile tissue, light buff in the hymenial layer. The hymenium is light buff in color, becoming slightly darker in drying to what you can see in the specimens. Asci 290–320 $\times$ 10–12 microns. Paraphyses filiform, very slightly enlarged at the tips. Spores smooth hyaline, ellipsoidal, 13–15 $\times$ 7–8 microns.

"The peculiar stalk varied in length on all the specimens, and when broken seemed to be largely made up of sand held together by mycelium, without any very evident development of solid fungus tissue within, but characteristic of each specimen, as they came to me."

This is the first record of the species from California. The first collection of this fungus received from Florida was sent by

Dr. Beardslee, who wrote: "I found them in January and February in New Smyrna [Florida]." As indicated above by Mr. West the fungus was collected by him near Gainesville (the type locality) also in January and February, 1931. The California specimen was collected February 24, 1932, so that all three collections from Florida and California were obtained in either January or February.

In the collections by Beardslee no mention was made of a stem which was doubtless broken off in the process of collecting but as indicated by Dr. Bonar the stem appears to be made up of columns of sand held together by strands of mycelium and may not after all be a true stem. He also mentions hairs on the outside of the apothecium but these again are merely strands of mycelium and not regarded as true hairs. The collection of this species in California greatly extends the range of distribution and it is hoped that these notes will stimulate other collectors to look for this little known species.—F. J. SEAVER.



SARCOSPHAERA FUNERATA

NOTES AND BRIEF ARTICLES

Encyclopédie Mycologique

Volume 1[1] of this work has just been received. This volume consists of 430 pages with 220 text figures and 35 plates in color, the plates containing 880 figures. The text figures consist of drawings of spores, cystidia, and other microscopic characters. The plates are beautifully done and the entire work appears to be a valuable contribution to our knowledge of this genus.

Announcement

Under the direction of Dr. G. P. Clinton, the writer completed a thesis with the following title: "THE GENERA OF FUNGI IMPERFECTI: North American Species And Hosts, With Particular Reference to Connecticut." The work was conducted at the Osborn Botanical Laboratory of Yale University, and at the Conn. Agricultural Experiment Station. The dissertation was presented to the Faculty of the graduate school of Yale University, in candidacy for the doctorate degree. The author intends to publish this work in the near future.

The present work attempts to bring about morphological unity in the definition of the various members of the group. It includes a brief history of the classification of the Fungi Imperfecti. The numerous genera shown to be synonyms have been compiled, as well as those which in part have perfect forms. It also includes a comprehensive key to all genera, including recent forms. The author's arrangement of genera is primarily one where a definite form can be located again with least effort. The writer uses conidial characters not at all for the definition of Families,—using such characters for the Genera alone. The definition of the Orders is based upon the type of fruiting body present, and its position in relation to the substratum. In the present work

[1] Heim, Roger.—Le genre Inocybe, précédé d'une introduction générale à l'etude des Agarics ochrosporés. Paul Lechevalier & Fils, 12, Rue de Tournon, Paris. 225 franks.

criteria for conidial sections can be uniformly applied to all Genera, irrespective to which larger group they naturally belong.

The writer also presents a detailed survey of the distribution of Fungi Imperfecti found in Connecticut. In addition an attempt is made to indicate, so far as recorded in literature, for each genus, all species found on Connecticut Hosts,—which hosts are also found elsewhere within North America. Thus the survey presented will serve as an indicator of potential invasion of Fungi Imperfecti for most North American hosts. The survey also shows precisely what hosts a species has been recorded as invading.

In describing the 1500 Genera the simplest possible descriptive terms have been used. Similar criteria for description were employed for genera irrespective of family or order relationships. The description for each genus is followed by the name of the perfect form for that genus, or for some of its species. On the same line where the genus, its number, author, and citation of original description are indicated, there appears also in "symbol formation" the classification for that genus. Knowing the salient characters of each generic group indicated by this symbol formation, the reader can by a mere glance at this formation learn the fundamental characters of the genus. Citations for the original description are followed by citations for later descriptions and for species of that particular genus. Also indicated are the generic names of synonyms of that genus.

Following the description of the genus are lists of species with hosts on which the species have been recorded found. Those species with hosts actually found in Connecticut have distribution data for the state. All lists are arranged alphabetically or chronologically.

The total "Generic Key" is itself made up of a number of separate "keys." These are so arranged as to insure accuracy with a minimum of effort.

The present work, then, should serve as a comprehensive reference from which either general or specific information concerning the Fungi Imperfecti can be obtained. Various means are provided for locating such desired information easily and speedily.

HAROLD B. BENDER